

sample using culture, but this technique is negatively affected by considerable turn around time and suboptimal sensitivity, specificity and predictive value. It also can require more extensive handling of the organism, which can be particularly undesirable for some organisms such as security sensitive biological agents.

[0008] Alternative diagnostic techniques available include the use of polymerase chain reaction (PCR), particularly in conjunction with culture. However, a problem with such a technique is a lack of trust in early positive PCR results in the absence of culture results or relevant clinical or physical symptoms. Another problem with such a technique is, like with culture, a lack of sensitivity and specificity when attempting to detect small quantities of microbial nucleic acid in a background of host nucleic acid.

[0009] Hence, there is a recognized need for rapid and reliable techniques for accurate diagnosis of bacteria, yeast organisms and/or filamentous fungi.

SUMMARY

[0009a] In one aspect, the present disclosure refers to a method of identifying, partially identifying or classifying at least one bacterium in a sample, said method comprising analysing at least a portion of a bacterial 16S rRNA gene or gene product from the sample for the presence or absence of at least one single nucleotide polymorphism (SNP), wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1; wherein the at least one bacterium in the sample is identified, partially identified or classified based on the presence or absence of the at least one SNP.

[0009b] In another aspect, the present disclosure refers to a method of identifying, partially identifying or classifying at least one bacterium in a sample, said method comprising analysing at least a portion of a bacterial 16S rRNA gene or gene product from the sample for the presence or absence of at least one single nucleotide polymorphism (SNP), wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1; wherein the at least one bacterium in the sample is identified, partially identified or classified based on the presence or absence of the at least one SNP.

[0009c] In another aspect, the present disclosure refers to a method of identifying, partially

identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising analysing the presence or absence of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product in a sample from the subject; wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1; wherein the presence or absence of said at least one SNP in the at least a portion of the 16S rRNA gene or gene product is used to identify, partially identify, classify or diagnose the bacterial infection in the subject.

[0009d] In another aspect, the present disclosure refers to a method of identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising analysing the presence or absence of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product in a sample from the subject; wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1; wherein the presence or absence of said at least one SNP in the at least a portion of the 16S rRNA gene or gene product is used to identify, partially identify, classify or diagnose the bacterial infection in the subject.

[0009e] In another aspect, the present disclosure refers to a use of a therapy or treatment agent in the manufacture of a medicament for treating a subject having a bacterial infection, wherein said use comprises: identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject according to the method as disclosed herein, to identify, partially identify, classify or diagnose the bacterial infection in the subject; and that said therapy or treatment agent is to be administered to the subject for treating the bacterial infection in the subject.

[0009f] In another aspect, the present disclosure refers to a method of identifying or classifying at least one bacterium in a sample, said method comprising analysing nucleic acid from the sample for at least one SNP in a bacterial 16S rRNA gene at a position corresponding to at least one of positions 273, 378, 412, 440, 647, and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1, wherein the at least one bacterium is identified based on the presence of the at least one SNP.

[0009g] In another aspect, the present disclosure refers to a method of identifying or classifying at least one bacterium in a sample, said method comprising analysing nucleic acid from the

sample for at least two SNPs in a bacterial 16S rRNA gene at a position corresponding to at least two of positions 273, 378, 412, 440, 488, 647, and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1, wherein the at least one bacterium is identified based on the presence of the at least one SNP.

[0009h] In another aspect, the present disclosure refers to a method of identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising assaying a biological sample obtained from the subject for a property of at least one bacterial 16S rRNA gene or gene product or a portion thereof, wherein said assay comprises detecting at least one single nucleotide polymorphism (SNP) in the at least one bacterial 16S rRNA gene or gene product or a portion thereof, wherein the at least one SNP in the bacterial 16S rRNA gene or gene product or a portion thereof is at a position corresponding to at least one of positions 273, 378, 412, 440, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1; wherein the at least one bacteria in the sample is identified, partially identified, classified or diagnosed based on the presence or absence of the at least one SNP.

[0009i] In another aspect, the present disclosure refers to a method of identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising assaying a biological sample obtained from the subject for a property of at least one bacterial 16S rRNA gene or gene product or a portion thereof, wherein said assay comprises detecting at least one single nucleotide polymorphism (SNP) in the at least one bacterial 16S rRNA gene or gene product or a portion thereof, wherein the at least one SNP in the bacterial 16S rRNA gene or gene product or a portion thereof is at least two SNPs at a position corresponding to at least two of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1; wherein the at least one bacteria in the sample is identified, partially identified, classified or diagnosed based on the presence or absence of the at least one SNP.

[0009j] In yet another aspect, the present disclosure refers to at least one isolated probe, tool or reagent capable of identifying, partially identifying, or classifying at least one bacteria in a sample, wherein the probe, tool or reagent is capable of binding, detecting or identifying the presence or absence of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product, wherein the at least one probe, tool or reagent comprises an oligonucleotide comprising a nucleotide sequence as set forth in at least one of SEQ ID NOs: 22, 25-28, 33-35 and 57, or comprises an oligonucleotide consisting of a nucleotide sequence as set forth in at least one of SEQ ID NOs: 16, 17, 21, 23, 24, 29, 30-32

and 56.

[0009k] In yet another aspect, the present disclosure refers to a method of identifying, partially identifying, or classifying at least one bacterium in a sample, said method comprising: combining with the sample the at least one isolated probe, tool or reagent as disclosed herein; and identifying, partially identifying, or classifying the at least one bacterium based on the presence or absence of at least one at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product, wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

[0009l] In yet another aspect, the present disclosure refers to a method of identifying, partially identifying, or classifying at least one bacterium in a sample, said method comprising: combining with the sample the at least one isolated probe, tool or reagent as disclosed herein; and identifying, partially identifying, or classifying the at least one bacterium based on the presence or absence of at least one at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product, wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

[0009m] In yet another aspect, the present disclosure refers to an array comprising more than one said isolated probe, tool or reagent as disclosed herein.

[0009n] In yet another aspect, the present disclosure refers to a biochip comprising a solid substrate and at least one isolated probe, tool or reagent as disclosed herein.

[0009o] In yet another aspect, the present disclosure refers to a kit or assay for classifying or identifying at least one bacterium in a sample, said kit or assay comprising: the at least one probe, tool or reagent as disclosed herein; the array as disclosed herein; and/or the biochip as disclosed herein.

[0009p] In yet another aspect, the present disclosure refers to a use of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product as an indicator for classifying, identifying or partially identifying at least one bacterium, yeast organism or filamentous fungi in a sample; wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding

to at least one of positions 273, 378, 412, 440, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

[0009q] In yet another aspect, the present disclosure refers to a use of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product as an indicator for classifying, identifying or partially identifying at least one bacterium, yeast organism or filamentous fungi in a sample; wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

[0009r] In yet another aspect, the present disclosure refers to a use of the at least one single nucleotide polymorphism (SNP) as disclosed herein, or the at least one isolated probe, tool or reagent as disclosed herein in identifying, partially identifying or classifying at least one bacterium in a sample, wherein said at least one bacterium is identified, partially identified or classified based on the presence or absence of at least one SNP in at least a portion of the 16S rRNA gene or gene product from the sample.

[0010] In various examples the present disclosure is predicated in part on high conservation of the 16S (Svedberg unit) ribosomal RNA (16S rRNA) gene between prokaryotes, including bacteria, and the high conservation of the 18S ribosomal RNA (18S rRNA) gene between yeast organisms and filamentous fungi, and also in part on the discovery of multiple single nucleotide polymorphisms (SNPs) within the 16S rRNA gene and 18S rRNA gene that may be useful in the identification and classification of microbes, particularly bacterial microbes, and yeast organisms and/or filamentous fungi in a sample.

[0011] Generally speaking, prokaryotes, including bacteria, contain 16S rRNA, which is a component of the 30S small subunit of the prokaryotic ribosome. The 16S rRNA is approximately 1,500 nucleotides in length and encoded by the 16S rRNA gene (also referred to as 16S rDNA), which is generally part of a co-transcribed operon also containing the 23S and 5S rRNA genes. Although the DNA sequence of the 16S rRNA genes (and thus the RNA sequence of the 16S rRNA molecules) is highly conserved between prokaryotes, there are regions of variation (Weisberg W.G., *et al.*, 1991).

[0012] Similarly, the 18S rRNA gene in yeast and filamentous fungi is the homologue of the 16S rRNA gene in prokaryotes. The 18S rRNA is a component of the 40S small eukaryotic ribosomal subunit. The DNA sequence of the 18S rRNA gene (and thus the RNA sequence of

the 18S rRNA molecules) is also highly conserved in yeast and filamentous fungi.

[0013] According to a first example of the disclosure, there is provided a method of

identifying, partially identifying or classifying at least one bacterium, yeast organism or filamentous fungi in a sample.

In one example, said method comprises analysing at least a portion of a bacterial 16S rRNA gene or gene product from the sample, or at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product from the sample, for the presence or absence of at least one single nucleotide polymorphism (SNP),

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 488, 647, and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1; or

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is in or corresponds to the 16S rRNA gene set forth in SEQ ID NO: 43; or

wherein the at least one bacterium, yeast organism or filamentous fungi in the sample is identified, partially identified or classified based on the presence or absence of the at least one SNP.

In another example, said method comprises analysing at least a portion of a bacterial 16S rRNA gene or gene product from the sample, or at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product from the sample, for the presence or absence of at least one single nucleotide polymorphism (SNP),

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;

wherein the at least one bacterium, yeast organism or filamentous fungi in the sample is identified, partially identified or classified based on the presence or absence of the at least one SNP.

[0014] According to a second example of the present disclosure, there is provided a method of identifying, partially identifying, classifying or diagnosing a bacterial, yeast organism or filamentous fungi infection in a subject.

In one example, said method comprises analysing the presence or absence of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product or in at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product in a sample from the subject;

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 488,

647, 653 of the 16S rRNA gene set forth in SEQ ID NO: 1; or

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is in or corresponds to the 16S rRNA gene set forth in SEQ ID NO: 43; or

wherein the presence or absence of said at least one SNP in the at least a portion of the 16S rRNA gene or gene product or in the at least a portion of the 18S rRNA gene or gene product is used to identify, partially identify, classify or diagnose the bacterial, yeast organism or filamentous fungi infection in the subject.

In another example, said method comprises analysing the presence or absence of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product or in at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product in a sample from the subject;

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;

wherein the presence or absence of said at least one SNP in the at least a portion of the 16S rRNA gene or gene product or in the at least a portion of the 18S rRNA gene or gene product is used to identify, partially identify, classify or diagnose the bacterial, yeast organism or filamentous fungi infection in the subject.

[0015] According to a third example of the present disclosure, there is provided method of treating a subject having a bacterial, yeast organism or filamentous fungi infection, said method comprising:

identifying, partially identifying, classifying or diagnosing a bacterial, yeast organism or filamentous fungi infection in a subject according to the method of the second example, to identify, partially identify, classify or diagnose the bacterial, yeast organism or filamentous fungi infection in the subject;

administering to the subject a therapy or treatment agent for treating the bacterial, yeast organism or filamentous fungi infection in the subject.

[0016] According to a fourth example of the present disclosure, there is provided at least one isolated probe, tool or reagent.

[0017] In one example of the fourth example, said at least one isolated probe, tool or reagent is capable of identifying, partially identifying, or classifying at least one bacteria, yeast organism or filamentous fungi in a sample, wherein the probe, tool or reagent is capable of binding, detecting or identifying the presence or absence of at least one single nucleotide

polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product or at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product,

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 488, 647, 653 of the 16S rRNA gene set forth in SEQ ID NO: 1; or

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is in or corresponds to the 16S rRNA gene set forth in SEQ ID NO: 43.

[0018] In another example of the fourth example, said at least one isolated probe, tool or reagent is capable of identifying, partially identifying, or classifying at least one bacteria, yeast organism or filamentous fungi in a sample, wherein the probe, tool or reagent is capable of binding, detecting or identifying the presence or absence of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product or at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product,

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

[0019] According to another example of the fourth example, said at least one isolated probe, tool or reagent is capable of discriminating between a sample that comprises at least one bacteria, yeast organism or filamentous fungi and a sample that does not comprise at least one bacteria, yeast organism or filamentous fungi, wherein the probe, tool or reagent is capable of binding, detecting or identifying the presence or absence of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product or at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product,

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 488, 647, 653 of the 16S rRNA gene set forth in SEQ ID NO: 1; or

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is in or corresponds to the 16S rRNA gene set forth in SEQ ID NO: 43.

[0020] According to another example of the fourth example, said at least one isolated probe, tool or reagent is capable of discriminating between a sample that comprises at least one bacteria, yeast organism or filamentous fungi and a sample that does not comprise at least one bacteria, yeast organism or filamentous fungi, wherein the probe, tool or reagent is capable of binding, detecting or identifying the presence or absence of at least one single nucleotide

polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product or at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product,

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

[0021] According to a fifth example of the present disclosure, there is provided a method of identifying, partially identifying, or classifying at least one bacterium, yeast organism or filamentous fungi in a sample.

In one example, said method comprises:

combining with the sample the at least one isolated probe, tool or reagent of the fourth example; and

identifying, partially identifying, or classifying the at least one bacterium, yeast organism or filamentous fungi based on the presence or absence of at least one at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product or at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product,

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 488, 647, 653 of the 16S rRNA gene set forth in SEQ ID NO: 1; or

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is in or corresponds to the 16S rRNA gene set forth in SEQ ID NO: 43.

In another example, said method comprises:

combining with the sample the at least one isolated probe, tool or reagent of the fourth example; and

identifying, partially identifying, or classifying the at least one bacterium, yeast organism or filamentous fungi based on the presence or absence of at least one at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product or at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product,

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

[0022] According to a sixth example of the present disclosure, there is provided an array

(especially a microarray) comprising more than one said isolated probe, tool or reagent of the fourth example.

[0023] According to a seventh example of the present disclosure, there is provided a biochip comprising a solid substrate and at least one isolated probe, tool or reagent of the fourth example.

[0024] According to an eighth example of the present disclosure, there is provided a kit or assay.

[0025] In one example of the eighth example, the kit or assay is for classifying or identifying at least one bacterium or at least one yeast organism or filamentous fungi in a sample, said kit or assay comprising: the at least one probe, tool or reagent of the fourth example; the array of the sixth example; and/or the biochip of the seventh example.

[0026] In another example of the eighth example, the kit or assay is capable of discriminating between a sample that comprises at least one bacteria, yeast organism or filamentous fungi and a sample that does not comprise at least one bacteria, yeast organism or filamentous fungi, wherein the kit or assay comprises the probe, tool or reagent of the fourth example.

[0027] According to a ninth example of the present disclosure, there is provided at least one single nucleotide polymorphism (SNP).

In one example, said at least one SNP is in at least a portion of a bacterial 16S rRNA gene or gene product or at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product for use as an indicator for classifying, identifying or partially identifying at least one bacterium, yeast organism or filamentous fungi in a sample;

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 488, 647, 653 of the 16S rRNA gene set forth in SEQ ID NO: 1; or

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is in or corresponds to the 16S rRNA gene set forth in SEQ ID NO: 43.

In another example, said at least one SNP is in at least a portion of a bacterial 16S rRNA gene or gene product or at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product for use as an indicator for classifying, identifying or partially identifying at least one bacterium, yeast organism or filamentous fungi in a sample;

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 488,

647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

[0028] According to a tenth example of the present disclosure, there is provided a use of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product or at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product as defined in the first example, or the at least one isolated probe, tool or reagent of the fourth example in identifying, partially identifying or classifying at least one bacterium, yeast organism or filamentous fungi in a sample, wherein said at least one bacterium, yeast organism or filamentous fungi is identified, partially identified or classified based on the presence or absence of at least one SNP in at least a portion of the 16S rRNA gene or gene product or of at least a portion of the 18S rRNA gene or gene product from the sample.

[0029] According to an eleventh example of the present disclosure, there is provided a kit comprising a probe, tool or reagent for use in the method of any one of the first, second, third or fifth examples of the present disclosure.

[0030] Further examples of the disclosure are provided below.

[0031] According to a twelfth example of the present disclosure, there is provided at least one single nucleotide polymorphism (SNP) in a 16S rRNA gene for use or when used in identifying at least one bacterium in a sample or classifying bacteria in the sample; or at least one single nucleotide polymorphism (SNP) in a 18S rRNA gene for use or when used in identifying at least one yeast organism or filamentous fungi in a sample or classifying a yeast organism or filamentous fungi in the sample.

[0032] According to a thirteenth example of the present disclosure, there is provided at least one probe, tool or reagent for use or when used in identifying at least one bacterium in a sample or classifying bacteria in the sample, said at least one probe, tool or reagent is capable of specifically binding, detecting or identifying at least a portion of a 16S rRNA gene in the sample containing at least one SNP; or at least one probe, tool or reagent for use or when used in identifying at least one yeast organism or filamentous fungi in a sample or classifying at least one yeast organism or filamentous fungi in the sample, said at least one probe, tool or reagent is capable of specifically binding, detecting or identifying at least a portion of a 18S rRNA gene in the sample containing at least one SNP.

[0033] According to a fourteenth example of the present disclosure, there is provided at least one probe, tool or reagent for use or when used in identifying a bacterium in a sample or classifying bacteria in the sample, said at least one probe, tool or reagent comprising an

oligonucleotide having a nucleotide sequence as set forth in any one of the of SEQ ID NOs:16-35, 56 or 57; or at least one probe, tool or reagent for use or when used in identifying at least one yeast organism or filamentous fungi in a sample or classifying at least one yeast organism or filamentous fungi in the sample, said at least one probe, tool or reagent comprising an oligonucleotide having a nucleotide sequence as set forth in any one of the of SEQ ID NOs:53-55.

[0034] According to a fifteenth example of the present disclosure, there is provided a use of the at least one SNP as defined in the twelfth example or the at least one probe, tool or reagent as defined in the thirteenth or fourteenth example in identifying at least one bacterium in a sample or classifying bacteria in the sample, wherein said at least one bacterium is identified or bacteria classified based on the presence of at least one SNP in a 16S rRNA gene from the sample as described above; or a use of the at least one SNP as defined in the twelfth example or the at least one probe, tool or reagent as defined in the thirteenth or fourteenth example in identifying at least one yeast organism or filamentous fungi in a sample or classifying at least one yeast organism or filamentous fungi in the sample, wherein said at least one yeast organism or filamentous fungi is identified or classified based on the presence of at least one SNP in a 18S rRNA gene from the sample as described above.

[0035] According to a sixteenth example of the present disclosure, there is provided a method of identifying at least one bacterium in a sample, said method comprising analysing a 16S rRNA gene from the sample for the at least one SNP as defined in the first example, wherein the at least one bacterium is identified based on the presence of the at least one SNP; or a method of identifying at least one yeast organism or filamentous fungi in a sample, said method comprising analysing a 18S rRNA gene from the sample for the at least one SNP as defined in the twelfth example, wherein the at least one yeast organism or filamentous fungi is identified based on the presence of the at least one SNP.

[0036] In one example there is provided a method of identifying at least one bacterium in a sample, said method comprising analysing nucleic acid from the sample for at least one SNP in a bacterial 16S rRNA gene at a position corresponding to at least one of positions 273, 378, 412, 440, 488, 647, and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1, wherein the at least one bacterium is identified based on the presence of the at least one SNP.

[0037] According to a seventeenth example of the present disclosure, there is provided a method of classifying bacteria in a sample, said method comprising analysing a 16S rRNA gene from the sample for the at least one SNP as defined in the first example, wherein the bacteria are

classified based on the presence of the at least one SNP; or a method of classifying at least one yeast organism or filamentous fungi in a sample, said method comprising analysing a 18S rRNA gene from the sample for the at least one SNP as defined in the twelfth example, wherein the at least one yeast organism or filamentous fungi are classified based on the presence of the at least one SNP.

[0038] According to an eighteenth example of the present disclosure, there is provided a method of identifying at least one bacterium in a sample, said method comprising:

combining with the sample the at least one probe, tool or reagent as defined in the thirteenth or fourteenth examples; and

identifying the at least one bacterium based on the presence of at least one SNP in a 16S rRNA gene from the sample as broadly described above; or

a method of identifying at least one yeast organism or filamentous fungi in a sample, said method comprising:

combining with the sample the at least one probe, tool or reagent as defined in the thirteenth or fourteenth examples; and

identifying the at least one yeast organism or filamentous fungi based on the presence of at least one SNP in a 18S rRNA gene from the sample as broadly described above.

[0039] According to a nineteenth example of the present disclosure, there is provided a method of classifying bacteria in a sample, said method comprising:

combining with the sample the at least one probe, tool or reagent as defined in the thirteenth or fourteenth examples; and

classifying the bacteria based on the presence of at least one SNP in a 16S rRNA gene from the sample as broadly described above; or

a method of classifying at least one yeast organism or filamentous fungi in a sample, said method comprising:

combining with the sample the at least one probe, tool or reagent as defined in the thirteenth or fourteenth examples; and

classifying the at least one yeast organism or filamentous fungi based on the presence of at least one SNP in a 18S rRNA gene from the sample as broadly described above.

[0040] According to a twentieth example of the present disclosure, there is provided a method of diagnosing a bacterial infection in a subject, said method comprising analysing a 16S rRNA gene from a sample obtained from the subject for the at least one SNP as defined in the twelfth

example, wherein the bacterial infection is diagnosed by identifying at least one causative bacterium in the sample based on the presence of the at least one SNP; or a method of diagnosing at least one yeast organism or filamentous fungi infection in a subject, said method comprising analysing a 18S rRNA gene from a sample obtained from the subject for the at least one SNP as defined in the twelfth example, wherein the at least one yeast organism or filamentous fungi infection is diagnosed by identifying at least one causative yeast organism or filamentous fungi in the sample based on the presence of the at least one SNP.

[0041] According to a twenty-first example of the present disclosure, there is provided a method of diagnosing a bacterial infection in a subject, said method comprising:

combining with a sample obtained from the subject the at least one probe, tool or reagent as defined in the thirteenth or fourteenth examples; and

diagnosing the bacterial infection by identifying at least one causative bacterium in the sample based on the presence of the at least one SNP in a 16S rRNA gene from the sample as broadly described above; or

a method of diagnosing at least one yeast organism or filamentous fungi infection in a subject, said method comprising:

combining with a sample obtained from the subject the at least one probe, tool or reagent as defined in the thirteenth or fourteenth examples; and

diagnosing the at least one yeast organism or filamentous fungi infection by identifying at least one causative bacterium in the sample based on the presence of the at least one SNP in a 18S rRNA gene from the sample as broadly described above.

[0042] According to a twenty-second example of the present disclosure, there is provided a method of treating a subject having a bacterial infection, said method comprising:

diagnosing the bacterial infection by identifying the at least one causative bacterium in the sample according to the method as defined in the twentieth or twenty-first examples; and

administering to the subject a therapy or treatment agent for treating the at least one causative bacterium identified to thereby treat the bacterial infection; or

a method of treating a subject having at least one yeast organism or filamentous fungi infection, said method comprising:

diagnosing the at least one yeast organism or filamentous fungi infection by identifying the at least one causative bacterium in the sample according to the method as defined in the twentieth or twenty-first examples; and

administering to the subject a therapy or treatment agent for treating the at least one

causative yeast organism or filamentous fungi identified to thereby treat the bacterial infection.

[0043] According to a twenty-third example of the present disclosure, there is provided an array of oligonucleotide probes for identifying at least one bacterium in a sample or classifying bacteria in the sample, said probes comprising oligonucleotides which hybridize to at least one SNP in a 16S rRNA gene in the sample as broadly described above; or an array of oligonucleotide probes for identifying at least one yeast organism or filamentous fungi in a sample or classifying at least one yeast organism or filamentous fungi in the sample, said probes comprising oligonucleotides which hybridize to at least one SNP in a 18S rRNA gene in the sample as broadly described above.

[0044] According to a twenty-fourth example of the present disclosure, there is provided a microarray comprising oligonucleotide probes for identifying at least one bacterium in a sample or classifying bacteria in the sample, said probes comprising oligonucleotides which hybridize to at least one SNP in a 16S rRNA gene in the sample as broadly described above; or a microarray comprising oligonucleotide probes for identifying at least one yeast organism or filamentous fungi in a sample or classifying at least one yeast organism or filamentous fungi in the sample, said probes comprising oligonucleotides which hybridize to at least one SNP in a 18S rRNA gene in the sample as broadly described above.

[0045] According to a twenty-fifth example of the present disclosure, there is provided a biochip comprising a solid substrate and at least one oligonucleotide probe for identifying at least one bacterium in a sample or classifying bacteria in the sample, said at least one probe comprising an oligonucleotide which hybridize to at least one SNP in a 16S rRNA gene in the sample as broadly described above; or a biochip comprising a solid substrate and at least one oligonucleotide probe for identifying at least one yeast organism or filamentous fungi in a sample or classifying at least one yeast organism or filamentous fungi in the sample, said at least one probe comprising an oligonucleotide which hybridize to at least one SNP in a 18S rRNA gene in the sample as broadly described above.

[0046] According to a twenty-sixth example of the present disclosure, there is provided a kit or assay for identifying at least one bacterium in a sample or classifying bacteria in the sample, said kit or assay comprising: the at least one probe, tool or reagent as described above; the array of oligonucleotide probes as described above; the microarray as described above; and/or the biochip as described above; or a kit or assay for identifying at least one yeast organism or filamentous fungi in a sample or classifying at least one yeast organism or filamentous fungi in the sample, said kit or assay comprising: the at least one probe, tool or reagent as described

above; the array of oligonucleotide probes as described above; the microarray as described above; and/or the biochip as described above.

[0047] According to a twenty-seventh example of the present disclosure, there is provided a method of identifying, partially identifying, classifying or diagnosing an infection in a subject. In one example of the twenty-seventh example, said infection is a bacterial, yeast organism or filamentous fungi infection, said method comprising assaying a biological sample obtained from the subject for a property of at least one bacterial 16S rRNA gene or gene product or a portion thereof or at least one yeast organism or filamentous fungi 18S rRNA gene or gene product or a portion thereof, wherein said assay comprises detecting at least one single nucleotide polymorphism (SNP) in the at least one bacterial 16S rRNA gene or gene product or a portion thereof or at least one yeast organism or filamentous fungi 18S rRNA gene or gene product or a portion thereof,

wherein the at least one SNP in the bacterial 16S rRNA gene or gene product or a portion thereof is at a position corresponding to at least one of positions 273, 378, 412, 440, 488, 647, and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1; or

wherein the at least one SNP in the bacterial 16S rRNA gene or gene product or a portion thereof is in or corresponds to the 16S rRNA gene set forth in SEQ ID NO: 43; or

wherein the at least one bacteria, yeast organism or filamentous fungi in the sample is identified, partially identified, classified or diagnosed based on the presence or absence of the at least one SNP.

In another example of the twenty-seventh example, said infection is a bacterial, yeast organism or filamentous fungi infection, said method comprising assaying a biological sample obtained from the subject for a property of at least one bacterial 16S rRNA gene or gene product or a portion thereof or at least one yeast organism or filamentous fungi 18S rRNA gene or gene product or a portion thereof, wherein said assay comprises detecting at least one single nucleotide polymorphism (SNP) in the at least one bacterial 16S rRNA gene or gene product or a portion thereof or at least one yeast organism or filamentous fungi 18S rRNA gene or gene product or a portion thereof,

wherein the at least one SNP in the bacterial 16S rRNA gene or gene product or a portion thereof is at a position corresponding to at least one of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;

wherein the at least one bacteria, yeast organism or filamentous fungi in the sample is identified, partially identified, classified or diagnosed based on the presence or absence of the at

least one SNP.

[0048] Features of the first to twenty-seventh examples of the present disclosure, where appropriate, may be as described below.

[0049] In one example, said at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is selected from SNPs at positions corresponding to at least one of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene as set forth in SEQ ID NO:1. In some examples, more than one SNP may be used in the methods of the present disclosure. For example, at least two SNPs, at least three SNPs, at least four SNPs, at least five SNPs, at least six SNPs, at least seven SNPs, at least eight SNPs, at least nine SNPs, at least ten SNPs, or even at least eleven SNPs may be used.

[0050] In one example, said at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is selected from SNPs at positions corresponding to positions 273, 378, 412, 440, 488, 647 and 653 of the 16S rRNA gene as set forth in SEQ ID NO:1. In some examples, more than one SNP may be used in the methods of the present disclosure. For example, at least two SNPs, at least three SNPs, at least four SNPs, at least five SNPs, at least six SNPs, or even at least seven SNPs may be used.

[0051] In another example, said at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product may be in or correspond to the 16S rRNA gene as set forth in SEQ ID NO: 43. The at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product set forth in SEQ ID NO: 43 may be at a position corresponding to at least one of positions 746, 764, 771, or 785 of the 16S rRNA gene set forth in SEQ ID NO: 43 (or positions 737, 755, 762, or 776 of the 16S rRNA gene as set forth in SEQ ID NO:1). At least one said SNP, at least two said SNPs, at least three said SNPs or at least four said SNPs may be used.

[0052] In another example, the at least one SNP in the at least a portion of the yeast organism or filamentous fungi 18S rRNA gene or gene product may be in or correspond to the 18S rRNA gene set forth in SEQ ID NO: 37. The at least one SNP in the at least a portion of the yeast organism or filamentous fungi 18S rRNA gene or gene product may be at a position corresponding to at least one of positions 343, 371, 388, 416, and 467 of the 18S rRNA gene set forth in SEQ ID NO: 37. At least one said SNP, at least two said SNPs, at least three said SNPs, at least four said SNPs or at least five said SNPs may be used.

[0053] In a further example, said method comprises analysing at least a portion of a

bacterial 16S rRNA gene or gene product from the sample, or at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product from the sample, for the presence or absence of:

single nucleotide polymorphisms in the bacterial 16S rRNA gene at a position corresponding to positions 273, 378, 412, 440, 488, 647, and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1; or

single nucleotide polymorphisms in the bacterial 16S rRNA gene a position corresponding to positions 746, 764, 771, and 785 of the 16S rRNA gene set forth in SEQ ID NO: 43; or

single nucleotide polymorphisms in the yeast organism or filamentous fungi 18S rRNA gene at a position corresponding to positions 343, 371, 388, 416, and 467 of the 18S rRNA gene set forth in SEQ ID NO: 37.

[0054] In a further example, said method comprises analysing at least a portion of a bacterial 16S rRNA gene or gene product from the sample, or at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product from the sample, for the presence or absence of:

single nucleotide polymorphisms in the at least a portion of the bacterial 16S rRNA gene or gene product at a position corresponding to at least four of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1; or

single nucleotide polymorphisms in the yeast organism or filamentous fungi 18S rRNA gene at a position corresponding to positions 343, 371, 388, 416, and 467 of the 18S rRNA gene set forth in SEQ ID NO: 37.

[0055] In some examples, the bacterium or bacteria is or are selected from among mammalian (e.g., human) associated bacteria, soil associated bacteria and water associated bacteria. In particular examples, the bacterium or bacteria may be a sepsis-associated bacterium or bacteria.

[0056] In some particular examples, the bacterium or bacteria is or are selected from among at least one of: *Acinetobacter* spp.; *Actinobaccillus* spp.; *Actinomadura* spp.; *Actinomyces* spp.; *Actinoplanes* spp.; *Aeromonas* spp.; *Agrobacterium* spp.; *Alistipes* spp.; *Anaerococcus* spp.; *Arthrobacter* spp.; *Bacillus* spp.; *Bacteroides* spp.; *Brucella* spp.; *Bulleidia* spp.; *Burkholderia* spp.; *Cardiobacterium* spp.; *Citrobacter* spp.; *Clostridium* spp.; *Cornyeibacterium* spp.; *Dermatophilus* spp.; *Dorea* spp.; *Enterobacter* spp.; *Enterococcus* spp.; *Erysipelothrix* spp.; *Escherichia* spp.; *Eubacterium* spp.; *Ewardsiella* spp.; *Faecalibacterium*

spp.; *Filifactor* spp.; *Finegoldia* spp.; *Flavobacterium* spp.; *Francisella* spp.; *Gallicola* spp.; *Haemophilus* spp.; *Helococcus* spp.; *Holdemania* spp.; *Hyphomicrobium* spp.; *Klebsiella* spp.; *Lactobacillus* spp.; *Legionella* spp.; *Listeria* spp.; *Methylobacterium* spp.; *Micrococcus* spp.; *Micromonospora* spp.; *Mobiluncus* spp.; *Moraxella* spp.; *Morganella* spp.; *Mycobacterium* spp.; *Neisseria* spp.; *Nocardia* spp.; *Paenibacillus* spp.; *Parabacteroides* spp.; *Pasteurella* spp.; *Peptoniphilus* spp.; *Peptostreptococcus* spp.; *Planococcus* spp.; *Planomicrobium* spp.; *Plesiomonas* spp.; *Porphyromonas* spp.; *Prevotella* spp.; *Propionibacterium* spp.; *Proteus* spp.; *Providentia* spp.; *Pseudomonas* spp.; *Ralstonia* spp.; *Rhodococcus* spp.; *Roseburia* spp.; *Ruminococcus* spp.; *Salmonella* spp.; *Sedimentibacter* spp.; *Serratia* spp.; *Shigella* spp.; *Solobacterium* spp.; *Sphingomonas* spp.; *Staphylococcus* spp.; *Stenotrophomonas* spp.; *Streptococcus* spp.; *Streptomyces* spp.; *Tissierella* spp.; *Vibrio* spp.; and *Yersinia* spp.

[0057] In some more particular examples, the bacterium or bacteria is or are selected from among at least one of: *Acinetobacter baumannii*; *Acinetobacter calcoaceticus*; *Bacteroides fragilis*; *Bacteroides vulgatus*; *Citrobacter freundii*; *Enterobacter aerogenes*; *Enterobacter cloacae*; *Enterococcus avium*; *Enterococcus faecalis*; *Enterococcus faecium*; *Escherichia coli*; *Klebsiella oxytoca*; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Staphylococcus hominis*; *Staphylococcus saprophyticus*; *Stenotrophomonas maltophilia*; *Streptococcus agalactiae*; *Streptococcus anginosus*; *Streptococcus constellatus*; *Streptococcus intermedius*; *Streptococcus milleri*; *Streptococcus mitis*; *Streptococcus mutans*; *Streptococcus oralis*; *Streptococcus pneumoniae*; *Streptococcus pyogenes*; *Streptococcus sanguinis*; and *Streptococcus sobrinus*.

[0058] In particular examples, the bacterium or bacteria is or are selected from among at least one of: *Acinetobacter calcoaceticus*; *Enterobacter aerogenes*; *Enterobacter cloacae*; *Enterococcus faecalis*; *Enterococcus faecium*; *Escherichia coli*; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Streptococcus agalactiae*; *Streptococcus pneumoniae*; and *Streptococcus pyogenes*.

[0059] In particular examples, the bacterium or bacteria is or are selected from among at least one of: *Acinetobacter calcoaceticus*; *Enterobacter aerogenes*; *Enterobacter cloacae*; *Enterococcus faecalis*; *Enterococcus faecium*; *Escherichia coli*; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Streptococcus agalactiae*; *Streptococcus pneumoniae*;

Streptococcus pyogenes; *Listeria monocytogenes*; *Clostridium perfringens*; *Corynebacterium jeikeium*; *Bacteroides fragilis*; *Neisseria meningitidis*; *Haemophilus influenzae*; *Salmonella sp.*; and *Staphylococcus epidermidis*. In another example, the bacterium or bacteria is or are selected from among at least one of: *Acinetobacter calcoaceticus*; *Enterobacter aerogenes*; *Enterobacter cloacae*; *Enterococcus faecalis*; *Enterococcus faecium*; *Escherichia coli*; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Streptococcus agalactiae*; *Streptococcus pneumoniae*; *Streptococcus pyogenes*; *Listeria monocytogenes*; *Clostridium perfringens*; *Corynebacterium jeikeium*; *Bacteroides fragilis*; *Neisseria meningitidis*; *Haemophilus influenzae*; *Salmonella sp.*; *Staphylococcus epidermidis*; *Bacillus anthracis*, *Clostridium botulinum*, *Yersinia pestis*, *Francisella tularensis*, *Vibrio cholerae*, and *Burkholderia pseudomallei*.

[0060] In one example the bacterium or bacteria is a Security Sensitive Biological Agent (SSBA). The SSBA may be a Tier 1 agent or a Tier 2 agent. Exemplary Tier 1 agents include one or more of: *Bacillus anthracis* (Anthrax), and *Yersinia pestis* (Plague). Exemplary Tier 2 agents include one or more of: *Clostridium botulinum* (botulism, especially toxin producing strains); *Francisella tularensis* (Tularaemia); *Salmonella Typhi* (typhoid), and *Vibrio cholerae* (especially Cholera serotypes O1 or O139). In one example the bacterium or bacteria is or are selected from among at least one of the group consisting of: *Bacillus anthracis*, *Clostridium botulinum*, *Yersinia pestis*, *Francisella tularensis*, *Vibrio cholerae*, and *Burkholderia pseudomallei*.

[0061] In one example the yeast organism may be selected from among at least one of: *Candida spp.*, *Cryptococcus spp* and *Rhodotorula spp*. Exemplary *Candida spp.* may include at least one of *Candida albicans*, *Candida tropicalis*, *Candida stellatoidea*, *Candida krusei*, *Candida parapsilosis*, *Candida glabrata*, *Candida guilliermondii*, *Candida viswanathii*, *Candida auris* and *Candida lusitaniae*. Exemplary *Cryptococcus spp.* may include at least one of *Cryptococcus neoformans* and *Cryptococcus gattii*. An exemplary *Rhodotorula spp.* may be *Rhodotorula mucilaginosa*.

[0062] In one example the filamentous fungi may be selected from at least one of: *Aspergillus spp.* and *Fusarium spp.* Exemplary *Fusarium spp.* may include at least one of *Fusarium solani*, *Fusarium oxysporum*, *Fusarium verticillioides*, *Fusarium proliferatum*, *Fusarium avenaceum*, *Fusarium bubigeum*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium langsethiae*, *Fusarium poae*, *Fusarium sporotrichioides*, *Fusarium tricinctum* and

Fusarium virguliforme; especially at least one of *Fusarium solani*, *Fusarium oxysporum*, *Fusarium verticillioides* and *Fusarium proliferatum*. Exemplary *Aspergillus* spp. may include at least one of *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus clavatus*, *Aspergillus lentulus*, *Aspergillus terreus*, and *Aspergillus nidulans*; especially at least one of *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus clavatus*; more especially *Aspergillus fumigatus*.

[0063] In one example, the yeast organism or filamentous fungi is at least one of the group consisting of: *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, *Fusarium* sp., *Aspergillus fumigatus*, and *Cryptococcus neoformans*.

[0064] In one example, the bacteria, yeast organism or filamentous fungi is a human pathogen.

[0065] In some examples, the methods of the present disclosure may be used to analyse blood from a subject with systemic inflammatory response syndrome (SIRS) to determine the origin of the SIRS (for example bacteria, yeast organism or filamentous fungi). In other examples, the methods of the present disclosure may be used to determine whether a subject has sepsis having a bacterial, yeast organism or filamentous fungi infectious origin. In both examples, the methods of the present disclosure may be used to determine the presence of, differentiate and/or identify bacteria, yeast organism or filamentous fungi present in the sample.

[0066] SIRS is an overwhelming whole body reaction that may have an infectious aetiology or non-infectious aetiology (i.e., infection-negative SIRS, or inSIRS). Sepsis is SIRS that occurs during infection. Sepsis in this instance is diagnosed by a clinician (when there is suspected infection) or through culture of an organism. Both SIRS and sepsis are defined by a number of non-specific host response parameters including changes in heart and respiratory rate, body temperature and white cell counts (Levy *et al.*, 2003; Reinhart *et al.*, 2012).

[0067] In some examples, the at least one SNP or at least one probe, tool or reagent may be used to classify bacteria in a sample as Gram-positive bacterium or bacteria or Gram-negative bacterium or bacteria.

[0068] For example, in some examples, the bacterium or bacteria may be classified as Gram-positive based on any one of the above SNPs, especially at at least one of positions 273, 378, 412, 440, 488, 647, and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1. In one such example, the bacteria or bacterium may be classified based on SNPs at positions corresponding to positions 273 and 653 of the 16S rRNA gene as set forth in SEQ ID NO:1, wherein the bacterium or bacteria is determined to be Gram-positive when there is an A at

position 273 and a T at position 653.

[0069] For example, in another example, the bacterium or bacteria may be classified as Gram-positive based on at least one SNP at a position corresponding to position 440 of the 16S rRNA gene as set forth in SEQ ID NO:1, wherein the bacterium or bacteria is determined to be Gram-positive when there is a T at position 440. Conversely, wherein the bacterium or bacteria is determined to be Gram-negative when there is not a T at position 440.

[0070] In yet other examples, the at least one SNP or at least one probe, tool or reagent may be used to classify groups of bacteria, yeast organisms or filamentous fungi in a sample.

[0071] For example, in some examples, the bacteria may be classified as belonging to a particular genus based on at least one SNP selected from the above SNPs. In one such example, the bacteria may be classified as belonging to a particular genus based on at least one SNP selected from SNPs at positions corresponding to positions 412 and 647 of the 16S rRNA gene as set forth in SEQ ID NO:1. For example, the bacterium or bacteria in a sample may be classified as belonging to the *Staphylococcus* genus when there is a T at position 412. For example, the bacterium or bacteria in a sample may be classified as belonging to the *Enterococcus* genus when there is a G at position 647.

[0072] In yet other examples, the at least one SNP or at least one probe, tool or reagent may be used to identify a bacterium in a sample as described above.

[0073] For example, the bacterium *Enterobacter cloacae* may be identified in a sample based on at least one SNP at a position corresponding to position 653 of the 16S rRNA gene as set forth in SEQ ID NO:1, wherein the bacterium *Enterobacter cloacae* is identified when there is a G at position 653.

[0074] For example, bacterium selected from *Streptococcus pneumoniae*, *Streptococcus agalactiae* and *Streptococcus pyogenes* may be identified in a sample based on SNPs at positions corresponding to positions 378 and 488 of the 16S rRNA gene as set forth in SEQ ID NO:1, wherein the bacterium is: *Streptococcus pneumoniae* when there is an A at position 378 and a T at position 488; *Streptococcus agalactiae* when there is an A at position 378 and an A 488; and *Streptococcus pyogenes* when there is a G at position 378 and an A at position 488.

[0075] For example, in one example, bacterium selected from among *Acinetobacter calcoaceticus*; *Enterobacter cloacae*; *Escherichia coli*; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Streptococcus agalactiae*; *Streptococcus pneumoniae*; and *Streptococcus pyogenes* may be identified in a sample based on SNPs at positions

corresponding to positions 273, 378, 412, 440, 488, 647 and 653 of the 16S rRNA gene as set forth in SEQ ID NO:1, wherein the bacterium is: *Acinetobacter calcoaceticus* when there is an A at positions 273, 440 and 647; *Enterobacter cloacae* when there is a G at position 653; *Escherichia coli* when there is a T at position 273 and a T at position 653; *Klebsiella pneumoniae* when there is a T at position 273, a C at positions 488 and 647 and an A at position 653; *Proteus mirabilis* when there is a C at positions 440 and 488 and a T at position 647; *Pseudomonas aeruginosa* when there is an A at position 440 and a T at position 647; *Streptococcus agalactiae* when there is an A at positions 378, 488 and 647; *Streptococcus pneumoniae* when there is T at positions 488 and 647; and *Streptococcus pyogenes* when there is G at position 378 and A at positions 488 and 647.

[0076] For example, in another example, bacterium selected from among *Acinetobacter calcoaceticus*; *Enterobacter cloacae*; *Escherichia coli*; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Streptococcus agalactiae*; *Streptococcus pneumoniae*; and *Streptococcus pyogenes* may be identified in a sample based on the presence of SNPs set forth in the following table:

Table 1

Bacterial species	SNP position in the 16S rRNA gene as set forth in SEQ ID NO:1						
	273	378	412	440	488	647	653
<i>Escherichia coli</i>	T	G	A	C	C	C	T
<i>Streptococcus pneumoniae</i>	A	A	A	T	T	T	T
<i>Streptococcus agalactiae</i>	A	A	A	T	A	A	T
<i>Streptococcus pyogenes</i>	A	G	A	T	A	A	T
<i>Proteus mirabilis</i>	T	G	A	C	C	T	A
<i>Enterobacter cloacae</i>	T	G	A	C	C	C	G
<i>Klebsiella pneumoniae</i>	T	G	A	C	C	C	A
<i>Pseudomonas aeruginosa</i>	A	G	A	A	C	T	A
<i>Acinetobacter calcoaceticus</i>	A	G	A	A	C	A	A

[0077] For example, in another example, bacterium selected from among *Escherichia coli*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Proteus mirabilis*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Corynebacterium jeikeium*, *Bacteroides fragilis*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Serratia marcescens*, *Salmonella sp.*, and *Staphylococcus epidermidis* may be identified in a sample based on the presence of SNPs set forth in the following table:

[0078] In another example, bacterium selected from among *Bacillus anthracis*, *Clostridium botulinum* type A, *Clostridium botulinum* type B, *Clostridium botulinum* type C, *Clostridium botulinum* type D, *Clostridium botulinum* type G, *Yersinia pestis*, *Francisella tularensis*, *Vibrio cholerae* and *Burkholderia pseudomallei* may be identified in a sample based on SNPs at positions corresponding to positions 746, 764, 771, or 785 of the 16S rRNA gene as set forth in SEQ ID NO:43, wherein the bacterium is: *Bacillus anthracis* when there is a T at position 746, A at position 764, C at position 771 and G at position 785; *Clostridium botulinum* type A or *Clostridium botulinum* type B when there is a T at position 746, G at position 764, C at position 771 and T at position 785; *Clostridium botulinum* type C when there is a T at position 746, A at position 764, T at position 771 and T at position 785; *Clostridium botulinum* type D when there is a C at position 746, A at position 764, T at position 771 and T at position 785; *Clostridium botulinum* type G when there is a T at position 746, G at position 764, C at position 771 and G at position 785; *Yersinia pestis* when there is a C at position 746, G at position 764, T at position 771 and G at position 785; *Francisella tularensis* when there is a T at position 746, A at position 764, G at position 771 and G at position 785; *Vibrio cholerae* when there is a C at position 746, A at position 764, T at position 771 and G at position 785; and *Burkholderia pseudomallei* when there is a C at position 746, G at position 764, C at position 771 and G at position 785.

[0079] For example, in another example, bacterium selected from among *Bacillus anthracis*, *Clostridium botulinum* type A, *Clostridium botulinum* type B, *Clostridium botulinum* type C, *Clostridium botulinum* type D, *Clostridium botulinum* type G, *Yersinia pestis*, *Francisella tularensis*, *Vibrio cholerae* and *Burkholderia pseudomallei* may be identified in a sample based on the presence of SNPs set forth in the following table:

Table 3

Bacterial species	SNP position in the 16S rRNA gene as set forth in SEQ ID NO: 43			
	746	764	771	785
<i>Bacillus anthracis</i>	T	A	C	G
<i>Clostridium botulinum</i> type A	T	G	C	T
<i>Clostridium botulinum</i> type B	T	G	C	T
<i>Clostridium botulinum</i> type C	T	A	T	T
<i>Clostridium botulinum</i> type D	C	A	T	T
<i>Clostridium botulinum</i> type G	T	G	C	G
<i>Yersinia pestis</i>	C	G	T	G
<i>Francisella tularensis</i>	T	A	G	G
<i>Vibrio cholerae</i>	C	A	T	G
<i>Burkholderia pseudomallei</i>	C	G	C	G

The cumulative discriminatory index of the four SNPs used to identify the above organisms are

0.667 for 1 SNP; 0.889 for 2 SNPs; 0.944 for 3 SNPs; and 0.972 for 4 SNPs.

[0080] Position 746 of the 16S rRNA gene set forth in SEQ ID NO:43 corresponds to position 737 of the 16S rRNA gene set forth in SEQ ID NO:1. Position 764 of the 16S rRNA gene set forth in SEQ ID NO:43 corresponds to position 755 of the 16S rRNA gene set forth in SEQ ID NO:1. Position 771 of the 16S rRNA gene set forth in SEQ ID NO:43 corresponds to position 762 of the 16S rRNA gene set forth in SEQ ID NO:1. Position 785 of the 16S rRNA gene set forth in SEQ ID NO:43 corresponds to position 776 of the 16S rRNA gene set forth in SEQ ID NO:1.

[0081] Therefore, in another example, bacterium selected from among *Bacillus anthracis*, *Clostridium botulinum type A*, *Clostridium botulinum type B*, *Clostridium botulinum type C*, *Clostridium botulinum type D*, *Clostridium botulinum type G*, *Yersinia pestis*, *Francisella tularensis*, *Vibrio cholerae* and *Burkholderia pseudomallei* may be identified in a sample based on SNPs at positions corresponding to positions 737, 755, 762, or 776 of the 16S rRNA gene as set forth in SEQ ID NO:1, wherein the bacterium is: *Bacillus anthracis* when there is a T at position 737, A at position 755, C at position 762 and G at position 776; *Clostridium botulinum type A* or *Clostridium botulinum type B* when there is a T at position 737, G at position 755, C at position 762 and T at position 776; *Clostridium botulinum type C* when there is a T at position 737, A at position 755, T at position 762 and T at position 776; *Clostridium botulinum type D* when there is a C at position 737, A at position 755, T at position 762 and T at position 776; *Clostridium botulinum type G* when there is a T at position 737, G at position 755, C at position 762 and G at position 776; *Yersinia pestis* when there is a C at position 737, G at position 755, T at position 762 and G at position 776; *Francisella tularensis* when there is a T at position 737, A at position 755, G at position 762 and G at position 776; *Vibrio cholerae* when there is a C at position 737, A at position 755, T at position 762 and G at position 776; and *Burkholderia pseudomallei* when there is a C at position 737, G at position 755, C at position 762 and G at position 776.

[0082] In another example, yeast organism or filamentous fungi selected from among *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, *Fusarium spp.*, *Aspergillus fumigatus*, and *Cryptococcus neoformans* may be identified in a sample based on SNPs at positions corresponding to positions 343, 371, 388, 416, and 467 of the 18S rRNA gene set forth in SEQ ID NO: 37, wherein the yeast organism or filamentous fungi is: *Candida albicans* when there is a C at position 343, A at position 371, T at position 388, G at position 416 and G at position 467; *Candida tropicalis* when there is a C at position 343, A at position

371, C at position 388, G at position 416 and C at position 467; *Candida parapsilosis* when there is a C at position 343, A at position 371, C at position 388, G at position 416 and G at position 467; *Candida glabrata* when there is a T at position 343, A at position 371, C at position 388, G at position 416 and G at position 467; *Fusarium* spp. when there is a C at position 343, C at position 371, T at position 388, T at position 416 and G at position 467; *Aspergillus fumigatus* when there is a C at position 343, C at position 371, T at position 388, C at position 416 and G at position 467; and *Cryptococcus neoformans* when there is a C at position 343, A at position 371, T at position 388, T at position 416 and G at position 467.

[0083] For example, in another example, yeast organism or filamentous fungi selected from among *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, *Fusarium* spp., *Aspergillus fumigatus*, and *Cryptococcus neoformans* may be identified in a sample based on the presence of SNPs set forth in the following table:

Table 4

Yeast organism or filamentous fungi species	SNP position in the 18S rRNA gene as set forth in SEQ ID NO: 37				
	343	371	388	416	467
<i>Candida albicans</i>	C	A	T	G	G
<i>Candida tropicalis</i>	C	A	C	G	C
<i>Candida parapsilosis</i>	C	A	C	G	G
<i>Candida glabrata</i>	T	A	C	G	G
<i>Fusarium</i> sp.	C	C	T	T	G
<i>Aspergillus fumigatus</i>	C	C	T	C	G
<i>Cryptococcus neoformans</i>	C	A	T	T	G

[0084] The bacteria, yeast organism or filamentous fungi may be partially identified or classified based on one or more of the above SNPs.

[0085] In one example nucleic acid is extracted from the sample prior to analysis in the methods of the disclosure (especially in the first and second examples). In another example, the step of analysing in the methods (especially in the first and second examples) may comprise amplification of nucleic acid. The nucleic acid may be amplified by any method known in the art including, but not limited to polymerase chain reaction (PCR), ligase chain reaction (LCR) and reverse transcription-polymerase chain reaction (RT-PCR) using one or more oligonucleotides/primers that will amplify transcribed RNA.

[0086] The SNPs may be analysed by any method known in the art including, but not limited to: high resolution melt analysis, 5' nuclease digestion (including 5' nuclease digestion), molecular beacons, oligonucleotide ligation, microarray, restriction fragment length polymorphism; antibody detection methods; direct sequencing or any combination thereof. In

one example, the step of analysing in the methods (especially in the first and second examples of the disclosure) comprises determining the presence or the absence of the at least one SNP using high resolution melt analysis, 5' nuclease digestion, molecular beacons, oligonucleotide ligation, microarray, restriction fragment length polymorphism, antibody detection methods; direct sequencing or any combination thereof. The SNPs may be detected by any method known in the art including, but not limited to: polymerase chain reaction (PCR); ligase chain reaction (LCR); hybridization analysis; high-resolution melt analysis; digestion with nucleases, including 5' nuclease digestion; molecular beacons; oligonucleotide ligations; microarray; restriction fragment length polymorphism; antibody detection methods; direct sequencing; or any combination thereof.

[0087] For example, in some examples, the identifying of bacterium or classifying of bacteria may further be based on DNA melting characteristics of the SNPs as broadly described above and their surrounding DNA sequences, preferably high-resolution melt analysis.

[0088] For example, in some such examples, the methods of the present disclosure may further include high-resolution melt (HRM) analysis to further analyse the DNA melting characteristics of the SNPs as broadly described above and their surrounding DNA sequences. In a particular example, the HRM analysis may include forming a DNA amplification product (i.e., amplicon) containing at least one of the SNPs and at least one intercalating fluorescent dye and heating the DNA amplification product through its melting temperature (T_m). The HRM is monitored in real-time using the fluorescent dye incorporated into the DNA amplification product. The level of fluorescence is monitored as the temperature increases with the the fluorescence reducing as the amount of double-stranded DNA reduces. Changes in fluorescence and temperature can be plotted in a graph known as a melt curve.

[0089] As a skilled addressee will understand, the T_m of the DNA amplification product at which the two DNA strands separate is predictable, being dependent on the sequence of the nucleotide bases forming the DNA amplification product. Accordingly, it is possible to differentiate between DNA amplification products including a DNA amplification product containing a polymorphism (i.e., a SNP or SNPs) as the melt curves will appear different. Indeed, in some examples, it is possible to differentiate between DNA amplification products containing the same polymorphism based on differences in the surrounding DNA sequences.

[0090] For example, bacterium selected from among *Acinetobacter calcoaceticus*; *Enterobacter aerogenes*; *Enterobacter cloacae*; *Enterococcus faecalis*; *Enterococcus faecium*;

Escherichia coli; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Streptococcus agalactiae*; *Streptococcus pneumoniae*; and *Streptococcus pyogenes* may be identified in a sample based on the presence of SNPs set forth in the following table and DNA melting characteristics of the SNPs and their surrounding DNA sequences:

Table 5

Bacterial species	SNP position in the 16S rRNA gene as set forth in SEQ ID NO:1						
	273	378	412	440	488	647	653
<i>Escherichia coli</i>	T	G	A	C	C	C	T
<i>Staphylococcus aureus</i>	A	G	T	T	C	A	T
<i>Staphylococcus epidermidis</i>	A	G	T	T	C	A	T
<i>Streptococcus pneumoniae</i>	A	A	A	T	T	T	T
<i>Streptococcus agalactiae</i>	A	A	A	T	A	A	T
<i>Streptococcus pyogenes</i>	A	G	A	T	A	A	T
<i>Enterococcus faecalis</i>	A	A	A	T	T	G	T
<i>Enterococcus faecium</i>	A	A	A	T	T	G	T
<i>Proteus mirabilis</i>	T	G	A	C	C	T	A
<i>Serratia marcescens</i>	T	G	A	C	T	C	A
<i>Enterobacter aerogenes</i>	T	G	A	C	T	C	A
<i>Enterobacter cloacae</i>	T	G	A	C	C	C	G
<i>Klebsiella pneumoniae</i>	T	G	A	C	C	C	A
<i>Pseudomonas aeruginosa</i>	A	G	A	A	C	T	A
<i>Acinetobacter calcoaceticus</i>	A	G	A	A	C	A	A

[0091] For example, bacterium selected from among *Escherichia coli*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Proteus mirabilis*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Corynebacterium jeikeium*, *Bacteroides fragilis*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Serratia marcescens*, *Salmonella sp.*, *Staphylococcus epidermidis* may be identified in a sample based on the presence of SNPs set forth in Table 2 and DNA melting characteristics of the SNPs and their surrounding DNA sequences.

[0092] In one example, bacteria selected from *Acinetobacter calcoaceticus*; *Enterobacter aerogenes*; *Enterobacter cloacae*; *Enterococcus faecalis*; *Enterococcus faecium*; *Escherichia coli*; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Streptococcus agalactiae*; *Streptococcus pneumoniae*; and *Streptococcus pyogenes* may be identified in a sample based on SNPs at positions corresponding to positions 273, 378, 412, 440, 488, 647 and 653 of the 16S rRNA gene as set forth in SEQ ID NO:1 and high-resolution melt curve analysis of the SNPs

and their surrounding DNA.

[0093] For example, the bacterium selected from among *Acinetobacter calcoaceticus*; *Enterobacter cloacae*; *Escherichia coli*; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Streptococcus agalactiae*; *Streptococcus pneumoniae*; and *Streptococcus pyogenes* may be identified in the sample based on the SNP positions as described above and/or high-resolution melt curve analysis of the SNPs and their surrounding DNA.

[0094] In some examples, the bacterium selected from *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Enterococcus faecalis*; *Enterococcus faecium*; *Serratia marcescens*; and *Enterobacter aerogenes* may be individually identified in a sample based on SNPs at positions corresponding to positions 412, 440, 488 and 647 of the 16S rRNA gene as set forth in SEQ ID NO:1, wherein: *Staphylococcus aureus* and *Staphylococcus epidermidis* may be identified when there is a T at position 412 and then further distinguished from one another based on high-resolution melt curve analysis of the DNA surrounding the SNP at position 412; *Enterococcus faecalis* and *Enterococcus faecium* may be identified when there is a G at position 647 and then further distinguished from one another based on high-resolution melt curve analysis of the DNA surrounding the SNP at position 647; *Serratia marcescens* and *Enterobacter aerogenes* may be identified when there is a C at positions 440 and 647 and a T at position 488 and may then be further distinguished from one another based on high-resolution melt curve analysis of the DNA surrounding the SNPs at any one of positions 440, 488 and 647.

[0095] In other examples, the bacterium selected from *Enterococcus faecalis*; *Enterococcus faecium*; *Streptococcus agalactiae*; and *Streptococcus pyogenes* may be identified in a sample based on at least one SNP at a position corresponding to position 378 of the 16S rRNA gene as set forth in SEQ ID NO:1 and high-resolution melt curve analysis of the DNA surrounding the SNP at position 378.

[0096] For example, bacterium selected from among *Bacillus anthracis*, *Clostridium botulinum* type A, *Clostridium botulinum* type B, *Clostridium botulinum* type C, *Clostridium botulinum* type D, *Clostridium botulinum* type G, *Yersinia pestis*, *Francisella tularensis*, *Vibrio cholerae* and *Burkholderia pseudomallei* may be identified in a sample based on the presence of SNPs set forth in the following table and DNA melting characteristics of the SNPs and their surrounding DNA sequences:

Table 6

Bacterial species	SNP position in the 16S rRNA gene as set forth in SEQ ID NO: 43			
	746	764	771	785
<i>Bacillus anthracis</i>	T	A	C	G
<i>Clostridium botulinum type A</i>	T	G	C	T
<i>Clostridium botulinum type B</i>	T	G	C	T
<i>Clostridium botulinum type C</i>	T	A	T	T
<i>Clostridium botulinum type D</i>	C	A	T	T
<i>Clostridium botulinum type G</i>	T	G	C	G
<i>Yersinia pestis</i>	C	G	T	G
<i>Francisella tularensis</i>	T	A	G	G
<i>Vibrio cholerae</i>	C	A	T	G
<i>Burkholderia pseudomallei</i>	C	G	C	G

[0097] As noted above, position 746 of the 16S rRNA gene set forth in SEQ ID NO:43 corresponds to position 737 of the 16S rRNA gene set forth in SEQ ID NO:1. Position 764 of the 16S rRNA gene set forth in SEQ ID NO:43 corresponds to position 755 of the 16S rRNA gene set forth in SEQ ID NO:1. Position 771 of the 16S rRNA gene set forth in SEQ ID NO:43 corresponds to position 762 of the 16S rRNA gene set forth in SEQ ID NO:1. Position 785 of the 16S rRNA gene set forth in SEQ ID NO:43 corresponds to position 776 of the 16S rRNA gene set forth in SEQ ID NO:1.

[0098] In one example, bacteria selected from *Bacillus anthracis*, *Clostridium botulinum type A*, *Clostridium botulinum type B*, *Clostridium botulinum type C*, *Clostridium botulinum type D*, *Clostridium botulinum type G*, *Yersinia pestis*, *Francisella tularensis*, *Vibrio cholerae* and *Burkholderia pseudomallei* may be identified in a sample based on SNPs at positions corresponding to positions 746, 764, 771, or 785 of the 16S rRNA gene as set forth in SEQ ID NO:43 (or positions 737, 755, 762, or 776 of the 16S rRNA gene as set forth in SEQ ID NO:1) and high-resolution melt curve analysis of the SNPs and their surrounding DNA.

[0099] For example, the bacterium selected from among *Bacillus anthracis*, *Clostridium botulinum type A*, *Clostridium botulinum type B*, *Clostridium botulinum type C*, *Clostridium botulinum type D*, *Clostridium botulinum type G*, *Yersinia pestis*, *Francisella tularensis*, *Vibrio cholerae* and *Burkholderia pseudomallei* may be identified in the sample based on the SNP positions as described above and/or high-resolution melt curve analysis of the SNPs and their surrounding DNA.

[00100] In another example, yeast organism or filamentous fungi selected from among *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, *Fusarium spp.*, *Aspergillus fumigatus*, and *Cryptococcus neoformans* may be identified in a sample based on the presence of SNPs set forth in the following table and DNA melting characteristics of the

SNPs and their surrounding DNA sequences:

Table 7

Yeast organism or filamentous fungi species	SNP position in the 18S rRNA gene as set forth in SEQ ID NO: 37				
	343	371	388	416	467
<i>Candida albicans</i>	C	A	T	G	G
<i>Candida tropicalis</i>	C	A	C	G	C
<i>Candida parapsilosis</i>	C	A	C	G	G
<i>Candida glabrata</i>	T	A	C	G	G
<i>Fusarium sp.</i>	C	C	T	T	G
<i>Aspergillus fumigatus</i>	C	C	T	C	G
<i>Cryptococcus neoformans</i>	C	A	T	T	G

[00101] In one example, yeast organism or filamentous fungi selected from *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, *Fusarium spp.*, *Aspergillus fumigatus*, and *Cryptococcus neoformans* may be identified in a sample based on SNPs at positions corresponding to positions 343, 371, 388, 416, and 467 of the 18S rRNA gene set forth in SEQ ID NO: 37 and high-resolution melt curve analysis of the SNPs and their surrounding DNA.

[00102] For example, the yeast organism or filamentous fungi selected from among *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, *Fusarium spp.*, *Aspergillus fumigatus*, and *Cryptococcus neoformans* may be identified in the sample based on the SNP positions as described above and/or high-resolution melt curve analysis of the SNPs and their surrounding DNA.

[00103] In some examples, nucleic acid may be extracted from the sample prior to analysis, identifying, classifying and/or diagnosing. Generally, the analysis, identifying, classifying and/or diagnosing may include amplification of the nucleic acid. In some examples, the analysis, identifying, classifying and/or diagnosing may further include administering a therapeutic agent to the subject, such as, e.g., an antibiotic. In another example, a method of treatment (for example as in the third example of the present disclosure) may further comprise the step of determining whether the at least one bacteria, yeast organism or filamentous fungi is resistant to a therapeutic agent.

[00104] In one example, any suitable sample may be used in the methods of the present disclosure. Exemplary samples may comprise sputum, saliva, blood, cerebrospinal fluid or urine samples.

[00105] The probe, tool or reagent may be, but is not limited to, an oligonucleotide, a primer, a nucleic acid, a polynucleotide, DNA, cDNA, RNA, a peptide or a polypeptide. These

may be, for example, single stranded or double stranded, naturally occurring, isolated, purified, chemically modified, recombinant or synthetic.

[00106] The probe, tool or reagent may be, but is not limited to, an antibody or other type of molecule or chemical entity capable of specifically binding, detecting or identifying at least a portion of a 16S rRNA gene or an 18S rRNA gene in a sample containing at least one SNP.

[00107] The probe, tool or reagent may be any number or combination of the above, and the number and combination will depend on a desired result to be achieved – e.g., detection of SNP at a genomic level (genotyping) or at the RNA transcription level.

[00108] The probe, tool or reagent may be isolated. The probe, tool or reagent may be detectably labelled. A detectable label may be included in an amplification reaction. Suitable labels include fluorochromes, e.g. fluorescein isothiocyanate (FITC), rhodamine, Texas Red, phycoerythrin, allophycocyanin, 6-carboxyfluorescein (6-FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE), 6-carboxy-X-rhodamine (ROX), 6-carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM) or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), radioactive labels, e.g. ^{32}P , ^{35}S , ^3H ; etc. The label may be a two stage system, where the amplified DNA is conjugated to biotin, haptens, etc. having a high affinity binding partner, e.g. avidin, specific antibodies, etc., where the binding partner is conjugated to a detectable label. The label may be conjugated to one or both of the primers. Alternatively, the pool of nucleotides used in the amplification is labeled, so as to incorporate the label into the amplification product.

[00109] In particular examples, the at least one probe, tool or reagent is for specifically binding, detecting or identifying of a SNP at the genomic level or transcription level, preferably the former.

[00110] In preferred examples, the at least one probe, tool or reagent is for specifically binding, detecting or identifying at least a portion of a 16S rRNA or a 18S rRNA gene or gene product in a sample containing at least one SNP as set forth in any one of Tables 1 to 7.

[00111] When identifying, partially identifying, classifying a bacterium, yeast organism or filamentous fungi, or identifying, partially identifying or diagnosing a bacterial, yeast organism or filamentous fungi infection a single probe (especially primer) may be used with each sample, or multiple probes (especially primers) may be used with each sample (i.e. in one pot). Such probes (especially primers) can be added to the raw solution obtained from amplification (such as PCR).

[00112] In one example, the at least one probe, tool or reagent may comprise two primers, each of which hybridizes to at least a portion of a bacterial 16S rRNA gene (or gene product) or to at least a portion of a yeast organism or filamentous fungi 18S rRNA gene (or gene product), containing a SNP as defined above. In another example, the at least one probe, tool or reagent may comprise a probe that hybridizes to at least a portion of a bacterial 16S rRNA gene or gene product or to at least a portion of a yeast organism or filamentous fungi 18S rRNA gene or gene product containing a SNP as defined above.

[00113] In one example, said at least one probe, tool or reagent comprises an oligonucleotide having (or comprising or consisting of) a nucleotide sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence homology or identity with the sequence as set forth in at least one of SEQ ID Nos 16-35 and 53-57. Said probe, tool or reagent may be a primer. Said probe, tool or reagent may comprise an oligonucleotide having a nucleotide sequence as set forth in at least one of SEQ ID NOs: 16-35 and 53-57.

[00114] In one example, the present disclosure provides at least one isolated probe, tool or reagent, wherein said at least one isolated probe, tool or reagent comprises an oligonucleotide having (or comprising or consisting of) a nucleotide sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence homology or identity with the sequence as set forth in at least one of SEQ ID Nos 16-35 and 53-57. Said probe, tool or reagent may be a primer.

[00115] Suitable primers for identification of SNPs in the 16S rRNA sequence set forth in SEQ ID NO. 43 (especially for identification of the SNPs in Table 3) may be as shown in the Table below.

Table 8

Forward primer sequence	Reverse primer sequence
5'GTGTAGCGGTGAAATGCGTAGAG 3' (SEQ ID NO.56)	5'TCGTTTACCGTGGACTACCAGGG 3' (SEQ ID NO. 57)

[00116] Suitable primers for identification of SNPs in the 18S rRNA sequence set forth in SEQ ID NO. 37 (especially for identification of the SNPs in Table 4) may be as shown in the Table below.

Table 9

Forward primer sequence	Reverse primer sequence
5'CATCCAAGGAAGGCAGCAGGCGCG 3' (SEQ ID NO. 53)	5'GTTCAACTACGAGCTTTTAAAC 3' (SEQ ID NO. 54)
	5'GTTGCGACTACGAGCTTTTAAAC 3' (SEQ ID NO. 55)

[00117] Any of the features described herein can be combined in any combination with any one or more of the other features described herein within the scope of the disclosure.

[00118] The above largely discusses the use of 16S rRNA and 18S rRNA genes. However, the above may also be applicable to 16S rRNA and to 18S rRNA and to other 16S rRNA and 18S rRNA gene products. Accordingly in some examples (and where appropriate), references to 16S rRNA gene and 18S rRNA gene above and below may be replaced with 16S rRNA gene product (or 16S rRNA) and 18S rRNA gene product (or 18S rRNA).

[00119] The reference to any prior art in this specification is not, and should not be taken as an acknowledgement or any form of suggestion that the prior art forms part of the common general knowledge.

BRIEF DESCRIPTION OF DRAWINGS

[00120] Various embodiments of the invention will be described with reference to the following drawings, in which:

[00121] Figure 1 is a CLUSTALW sequence alignment of the representative genes encoding 16S rRNA molecules from the following bacterial species: *Acinetobacter calcoaceticus*; *Enterobacter aerogenes*; *Enterobacter cloacae*; *Enterococcus faecalis*; *Enterococcus faecium*; *Escherichia coli*; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Streptococcus agalactiae*; *Streptococcus pneumoniae*; and *Streptococcus pyogenes*. Variable sequences, as determined by the CLUSTALW alignment were removed. The SNPs at positions corresponding to positions 273, 378, 412, 440, 488, 647 and 653 of the 16S rRNA gene from *E. coli* as set forth in SEQ ID NO:1 are highlighted together with the corresponding nucleotide in the aligned sequences.

[00122] Figure 2 shows a normalised high-resolution melt (HRM) curves plot for the following 15 bacterial species tested in Example 1: *Acinetobacter calcoaceticus*; *Enterobacter aerogenes*; *Enterobacter cloacae*; *Enterococcus faecalis*; *Enterococcus faecium*; *Escherichia coli*; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Streptococcus agalactiae*;

CLAIMS

1. A method of identifying, partially identifying or classifying at least one bacterium in a sample, said method comprising analysing at least a portion of a bacterial 16S rRNA gene or gene product from the sample for the presence or absence of at least one single nucleotide polymorphism (SNP),

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;

wherein the at least one bacterium in the sample is identified, partially identified or classified based on the presence or absence of the at least one SNP.

2. A method of identifying, partially identifying or classifying at least one bacterium in a sample, said method comprising analysing at least a portion of a bacterial 16S rRNA gene or gene product from the sample for the presence or absence of at least one single nucleotide polymorphism (SNP),

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;

wherein the at least one bacterium in the sample is identified, partially identified or classified based on the presence or absence of the at least one SNP.

3. A method of identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising analysing the presence or absence of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product in a sample from the subject;

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;

wherein the presence or absence of said at least one SNP in the at least a portion of the 16S rRNA gene or gene product is used to identify, partially identify, classify or diagnose the bacterial infection in the subject.

4. A method of identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising analysing the presence or absence of at least one

single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product in a sample from the subject;

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;

wherein the presence or absence of said at least one SNP in the at least a portion of the 16S rRNA gene or gene product is used to identify, partially identify, classify or diagnose the bacterial infection in the subject.

5. Use of a therapy or treatment agent in the manufacture of a medicament for treating a subject having a bacterial infection, wherein said use comprises:

identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject according to the method of claim 3 or claim 4, to identify, partially identify, classify or diagnose the bacterial infection in the subject; and

that said therapy or treatment agent is to be administered to the subject for treating the bacterial infection in the subject.

6. The method of any one of claims 1 to 4 or the use of claim 5, wherein said method or use comprises analysing at least a portion of a bacterial 16S rRNA gene or gene product from the sample for the presence or absence of:

single nucleotide polymorphisms in the at least a portion of the bacterial 16S rRNA gene or gene product at a position corresponding to at least four of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

7. A method of identifying or classifying at least one bacterium in a sample, said method comprising analysing nucleic acid from the sample for at least one SNP in a bacterial 16S rRNA gene at a position corresponding to at least one of positions 273, 378, 412, 440, 647, and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1, wherein the at least one bacterium is identified based on the presence of the at least one SNP.

8. A method of identifying or classifying at least one bacterium in a sample, said method comprising analysing nucleic acid from the sample for at least two SNPs in a bacterial 16S rRNA gene at a position corresponding to at least two of positions 273, 378, 412, 440, 488, 647, and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1, wherein the at least one bacterium is identified based on the presence of the at least one SNP.

9. The method of any one of claims 1 to 4 or 6 to 8 or the use of claims 5 or 6, wherein the

bacterium is or the bacterial infection is caused by at least one of the group consisting of: *Acinetobacter calcoaceticus*; *Enterobacter aerogenes*; *Enterobacter cloacae*; *Enterococcus faecalis*; *Enterococcus faecium*; *Escherichia coli*; *Klebsiella pneumoniae*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Staphylococcus aureus*; *Streptococcus agalactiae*; *Streptococcus pneumoniae*; *Streptococcus pyogenes*; *Listeria monocytogenes*; *Clostridium perfringens*; *Corynebacterium jeikeium*; *Bacteroides fragilis*; *Neisseria meningitidis*; *Haemophilus influenzae*; *Salmonella sp.*; and *Staphylococcus epidermidis*.

10. The method of any one of claims 1 to 4, 6 to 9 or the use of any one of claims 5, 6, or 9, wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

11. The method or use of claim 10, wherein the bacterium is or the bacterial infection is caused by at least one of the group consisting of: *Bacillus anthracis*, *Clostridium botulinum*, *Yersinia pestis*, *Francisella tularensis*, *Vibrio cholerae*, and *Burkholderia pseudomallei*.

12. The method of any one of claims 1 to 4 or 6 to 11, or the use of any one of claims 5, 6, or 9 to 11, wherein the sample is a biological sample comprising sputum, blood, cerebrospinal fluid or urine.

13. The method of any one of claims 1 to 4 or 6 to 12, or the use of any one of claims 5, 6, or 9 to 12, wherein the step of analysing comprises determining the presence or the absence of the at least one SNP using high resolution melt analysis, 5' nuclease digestion, molecular beacons, oligonucleotide ligation, microarray, restriction fragment length polymorphism, antibody detection methods, direct sequencing or any combination thereof.

14. The method of any one of claims 1 to 4 or 6 to 13, or the use of any one of claims 5, 6 or 9 to 13, wherein said method or use further comprises the step of determining whether the at least one bacteria is resistant to a therapeutic agent.

15. A method of identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising assaying a biological sample obtained from the subject for a property of at least one bacterial 16S rRNA gene or gene product or a portion thereof, wherein said assay comprises detecting at least one single nucleotide polymorphism (SNP) in the at least one bacterial 16S rRNA gene or gene product or a portion thereof,

wherein the at least one SNP in the bacterial 16S rRNA gene or gene product or a portion thereof is at a position corresponding to at least one of positions 273, 378, 412, 440, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;

wherein the at least one bacteria in the sample is identified, partially identified,

classified or diagnosed based on the presence or absence of the at least one SNP.

16. A method of identifying, partially identifying, classifying or diagnosing a bacterial infection in a subject, said method comprising assaying a biological sample obtained from the subject for a property of at least one bacterial 16S rRNA gene or gene product or a portion thereof, wherein said assay comprises detecting at least one single nucleotide polymorphism (SNP) in the at least one bacterial 16S rRNA gene or gene product or a portion thereof,

wherein the at least one SNP in the bacterial 16S rRNA gene or gene product or a portion thereof is at least two SNPs at a position corresponding to at least two of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;

wherein the at least one bacteria in the sample is identified, partially identified, classified or diagnosed based on the presence or absence of the at least one SNP.

17. At least one isolated probe, tool or reagent capable of identifying, partially identifying, or classifying at least one bacteria in a sample, wherein the probe, tool or reagent is capable of binding, detecting or identifying the presence or absence of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product,

wherein the at least one probe, tool or reagent comprises an oligonucleotide comprising a nucleotide sequence as set forth in at least one of SEQ ID NOs: 22, 25-28, 33-35 and 57, or comprises an oligonucleotide consisting of a nucleotide sequence as set forth in at least one of SEQ ID NOs: 16, 17, 21, 23, 24, 29, 30-32 and 56.

18. The at least one isolated probe, tool or reagent of claim 17, wherein the at least one probe, tool or reagent comprises an oligonucleotide comprising a nucleotide sequence as set forth in at least one of SEQ ID NOs: 22, 25-28, 33-35 and 57.

19. A method of identifying, partially identifying, or classifying at least one bacterium in a sample, said method comprising:

combining with the sample the at least one isolated probe, tool or reagent of claim 17 or claim 18; and

identifying, partially identifying, or classifying the at least one bacterium based on the presence or absence of at least one at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product,

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

20. A method of identifying, partially identifying, or classifying at least one bacterium in a

sample, said method comprising:

combining with the sample the at least one isolated probe, tool or reagent of claim 17 or claim 18; and

identifying, partially identifying, or classifying the at least one bacterium based on the presence or absence of at least one at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product,

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

21. An array comprising more than one said isolated probe, tool or reagent of claim 17 or claim 18.

22. A biochip comprising a solid substrate and at least one isolated probe, tool or reagent of claim 17 or claim 18.

23. A kit or assay for classifying or identifying at least one bacterium in a sample, said kit or assay comprising: the at least one probe, tool or reagent of claim 17 or claim 18; the array of claim 21; and/or the biochip of claim 22.

24. Use of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product as an indicator for classifying, identifying or partially identifying at least one bacterium, yeast organism or filamentous fungi in a sample;

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at a position corresponding to at least one of positions 273, 378, 412, 440, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

25. Use of at least one single nucleotide polymorphism (SNP) in at least a portion of a bacterial 16S rRNA gene or gene product as an indicator for classifying, identifying or partially identifying at least one bacterium, yeast organism or filamentous fungi in a sample;

wherein the at least one SNP in the at least a portion of the bacterial 16S rRNA gene or gene product is at least two SNPs at a position corresponding to at least two of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

26. Use of the at least one single nucleotide polymorphism (SNP) of claim 24 or 25, or the at least one isolated probe, tool or reagent of claim 17 or claim 18 in identifying, partially identifying or classifying at least one bacterium in a sample, wherein said at least one bacterium is identified, partially identified or classified based on the presence or absence of at least one

SNP in at least a portion of the 16S rRNA gene or gene product from the sample.

27. The method of any one of claims 1, 3, 15 and 19, wherein at least a portion of a bacterial 16S rRNA gene or gene product is analysed, and wherein the at least one SNP is at a position corresponding to at least one of positions 273, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

28. The method of any one of claims 2, 4, 16 and 20, wherein at least a portion of a bacterial 16S rRNA gene or gene product is analysed, and wherein the at least one SNP is at least five SNPs at a position corresponding to at least five of positions 273, 378, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1.

29. The method of claim 7, wherein the at least one SNP is at a position corresponding to at least one of positions 273, 647 and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1.

30. The method of claim 8, wherein the at least two SNPs are at least five SNPs at a position corresponding to at least five of positions 273, 378, 412, 440, 488, 647 and 653 of the 16S rRNA gene set forth in SEQ ID NO: 1.